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The linear quadratic fit for lung function after irradiation with X-rays at smaller doses per fraction than 2 Gy

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Preliminary results are given of an experiment to measure the repair capacity of mouse lungs after X-ray doses of 0.15 to 1.8 Gy per fraction.

The lung is relatively radiosensitive but it can be spared from X-ray damage by fractionating the irradiation (Field *et al.*, 1976; Travis *et al.*, 1983*a*; Vegesna *et al.*, 1985). Much less sparing occurs after neutron irradiation. The question is whether the sparing after X-irradiation is also observed for lower doses per fraction, smaller than the 2 Gy used in conventional radiotherapy. The linear quadratic (LQ) model for dose-response relationships has been found to fit many tissues reasonably well for doses per fraction down to 2 or 3 Gy. In spinal cord however Ang *et al.* (1985) observed no further sparing as doses per fraction decreased below 2 Gy: i.e. the total isoeffect dose did not increase as the size of fractions decreased further.

Radiation-induced changes in lung function are delayed until several months after irradiation of the mouse thorax and can be measured as an increase in breathing rate (Travis *et al.*, 1979). Experiments using multiple equal fractions of X-rays previously shown a good fit of data to the LQ model, with no change in the α/β values of $\sim$3 Gy, from single doses of 11 Gy down to 1 Gy per fraction of X-rays (40 fractions) (Travis *et al.*, 1983*a*; Parkins *et al.*, 1985; Parkins & Fowler, 1985). The results of Vegesna *et al.* (1985) do not disagree but suffered from the problem of incomplete repair in the short intervals used between fractions. The values of α/β for X-rays were $\sim$3 Gy for pneumonitis (4–7 months) and slightly, but not significantly, lower for the later phase (fibrosis?). For neutrons α/β was $\sim$30 Gy indicating the much larger non-reparable component of damage.

If the LQ model is valid, additional sparing should continue to be measurable down to about $0.1\,\alpha/\beta$ Gy, i.e. 0.3 Gy per fraction (Fowler *et al.*, 1983; Tucker & Thames, 1983). If multiple equal fractions were to be used to test this hypothesis, over 150 fractions would be required. This is not feasible without using intervals so short that repair of sublethal injury would be incomplete. In present experiments the top-up technique was used to investigate the effect of 20 equal fractions ranging from 0.15 to 1.4 Gy each (Denekamp, 1973; Travis *et al.*, 1983*b*). Graded top-up doses of neutrons are added so as to express the damage inflicted by the 20 small doses, which itself would be too small to measure. Neutrons were used for the top-up doses in order to minimize the effect of any changes in cell cycle distribution caused by the fractionated partial treatment (Joiner & Denekamp, 1985). The neutrons produced by the 4 MeV Van de Graaff accelerator at the Gray Lab (mean energy 3 MeV) are ideal for this purpose, having almost linear dose-response characteristics as indicated by the large α/β value. The resolution of the top-up method is best when more than half of the damage is delivered by the multifraction 'priming' doses under investigation. Clearly the resolution will be limited in an experiment where only 20 out of a full 150 fractions were given before the top-up dose. However, the present results will be reported because they represent an extension to lower doses per fraction than the previously published data on lung.

Materials and methods

The methods are fully described by Parkins *et al.* (1985). Briefly, the whole thorax of CBA/Ht Gy f BSVS mice aged 8–12 weeks (22–30 g) was irradiated with either 240 kVp X-rays or 3 MeV neutrons obtained from the 4 MeV Van de Graaff accelerator (Maughan *et al.*, 1981). The mice were held unanaesthetised in a perspex jig with only the thorax region unshielded. The breathing rate of the mice was measured at monthly intervals after 16 weeks post-irradiation using a whole-body plethysmograph (Travis *et al.*, 1979). The dose-related increase in breathing rate enabled dose-response curves to be constructed for each fractionation schedule.

The twenty fractions (the 'priming doses') were given as two fractions per day with a 6 h interval, with no weekend gap. The single ('top-up') test dose of neutrons was given one day after the last X-ray dose in order to allow repair of sublethal damage from the last fraction to be complete. A range of neutron doses was used so as to obtain a neutron dose-response curve for each X-ray priming

schedule. Two reference dose-response curves were also obtained, one for single doses of neutrons alone and the other for 20 equal fractions of X-rays without top-up, in order to define the limits and compare the results with previous experiments.

Results

Results can be expressed either as mean breathing rate in each dose group, plotted against radiation dose (the 'breathing rate' method) and analysed at an isoeffect level of increased breathing rate; or as the proportion of mice in each group which exceed 1.2 times the breathing rate of the sham (unirradiated) group (the 'ED_{50}' method). In addition lethality data were obtained (the 'LD_{50}' method). In Figure 1 the first presentation is shown, although all three methods were employed in the analysis below. The upper panel shows the effect of either single doses of neutrons alone or 20 equal fractions of X-rays. These curves represent the extremes of 100% top-up and zero top-up doses.

The lower panel in Figure 1 (note the different dose scale) shows the effect of 20 priming doses of X-rays ranging in size from 0.15 to 1.4 Gy per fraction, plotted against neutron top-up dose. It can be seen that 20 fractions of 0.15 Gy have inflicted measurable damage, because the dose-response curve is displaced to the left of that for no priming doses, i.e. the single dose neutron curve. As the size of the X-ray priming doses is increased, the neutron top-up curves shift to the left implying that more damage from the multiple X-ray fractions was 'remembered' at the time the top-up doses were given. The shift to the left shows that the effect of each fractionated schedule is resolvable from its neighbours.

The data can be interpreted most simply by taking the difference between the neutron top-up doses for no priming X-rays and for a given X-ray schedule as representing the damage caused by that schedule. In Figure 2 such a graph has been constructed (with an inverted vertical axis representing increased damage downwards). The effect of 20 fractions of 1.8 Gy of X-rays is equal to that of a neutron dose alone of about 7 Gy. A small correction has been applied to take account of the slight non-linearity of the neutron dose-response curve itself (Joiner & Denekamp, 1985); here $\alpha/\beta = 30$ Gy (Parkins *et al.*, 1985). The multiple points for each dose group represent up to 5 assay times between 4 and 11 months after irradiation for the three endpoints: (i) the dose at which the breathing rate increased to either 370 or 380 breaths per minute; (ii) the dose at which 50% of the mice exceed 1.2 × the breathing rate of the sham-

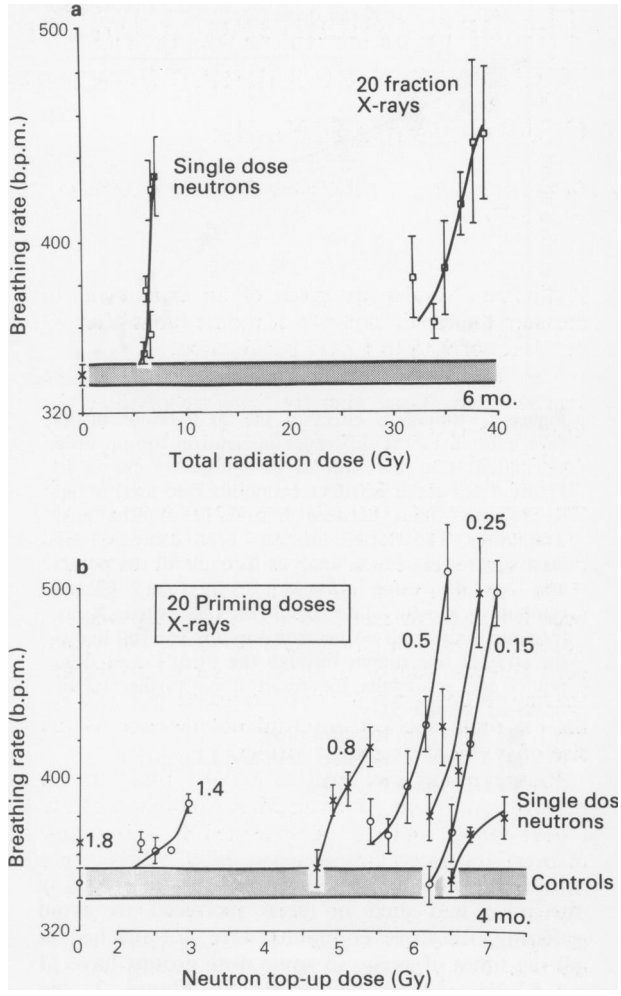

Figure 1 (a) Breathing rate as a function of total dose for (on left) single doses of neutrons only and (on right) 20 equal fractions of X-rays only, assayed at 6 months post irradiation. These represent 100% and zero top-up dose respectively, i.e. zero and 100% X-ray priming dose. The mean breathing rate ±1 s.e.m. of each group of 4 mice is plotted. The hatched area represents the breathing rate range of sham-irradiated control mice.

(b) Dose-response curves for ranges of single neutron top-up doses given to groups of mice irradiated with 20 equal fractions of X-rays (assayed at 4 months). The effect of increasing the X-ray priming dose is to shift the curves to the left because less neutron top-up dose is required. Resolved differences are seen between 0 and 0.15 Gy and between 0.15 and 0.25 Gy per fraction of X-rays. When the X-ray dose per fraction rises to 1.8 Gy, no neutron top-up dose is required. The pattern of shift across the page depends upon the shape of the X-ray dose-response curve, assuming equal effects per fraction.

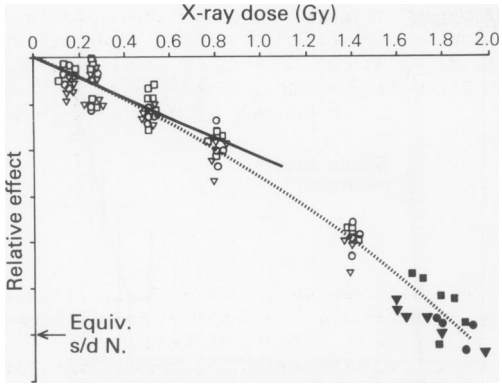

Figure 2 Biological effect of the 20 fractions of X-rays, plotted as the difference in neutron top-up dose calculated from the shift in dose-response curves in **Figure 1** for three isoeffect endpoints (see text) at up to 5 assay times between 6 and 12 months post irradiation. The dotted line has been computed by least squares regression analysis through all the points and has the value $\alpha/\beta = 3.1 \pm 0.5$ Gy (s.e.m.) Closed symbols represent data from the 20 equal X-ray fractions only, with no neutron top-up. The full line is the straight line drawn through the three lowest dose points and represents the result if no further repair occurred below 0.5 Gy.
(○, ●) 380 b.p.m. isoeefect dose;
(□, ■) 370 b.p.m. isoeffect dose and LD_{50};
(▽, ▼) 1.2 × sham b.r. ED_{50}.

irradiated controls; (iii) the dose at which 50% of the mice had died or been sacrificed to avoid suffering. All three endpoints were not reached at all the times of assay, so some dose groups have 11 or 13 instead of 15 points in Figure 2. No systematic trends of difference were seen between the relative results for these three end-points in this experiment.

It is interesting that significant damage can be measured, i.e., significantly different from zero damage, even at the lowest dose per fraction of 0.15 Gy.

The important question is whether the average values of effect, as assayed by the difference in top-up doses, conform to the linear quadratic model or not. All the data points have been fitted by least squares regression with an $(\alpha d + \beta d^2)$ curve which was found to have $\alpha/\beta = 3.1 \pm 0.5$ Gy (s.e.m.). This is clearly in good agreement with the previous results for mouse lung and supports the hypothesis that the response of lung to fractionated radiation is consistent with the LQ model, down to the lowest doses per fraction used, 0.15 Gy. However, the *relative* resolution is clearly poorer at the lower doses per fraction and the question about continually increasing repair as dose per fraction decreases cannot be resolved below 0.5 Gy by the present experiment.

If there were no further repair as dose per fraction was reduced, all the points would lie on a straight line. This is clearly not true for 1.8 and 1.4 Gy per fraction. Further, the 0.8 Gy points do not lie equally above and below the best straight line drawn through the 0.15, 0.25 and 0.5 Gy points. It cannot be excluded that the latter three dose groups may lie in a single straight line. Thus we cannot exclude a lack of further repair below 0.5 Gy, although the data are also consistent with a fit to the LQ model. It is likely but not certain that repair continues to increase down to at least 0.8 Gy. It is certain that repair is still increasing below 1.4 Gy per fraction.

Conclusions

The effect of multiple X-ray dose schedules too small to cause measurable damage by themselves can be investigated using the top-up techniques. The shape of the neutron top-up radiation response curve must be known (from previous multi-equal-fraction experiments) to correct for the not quite linear relationship to dose. The present results demonstrate that significant effects can be measured after twenty priming X-ray doses as small as 0.15 or 0.25 Gy per fraction. It would be an advantage to carry out top-up experiments with more than 20 fractions of this size in order to improve the resolution. The present results are consistent with the LQ model remaining valid down to such small doses per fraction, the same value of α/β (~ 3 Gy) being found as in previous multi-equal-dose experiments using higher doses per fraction. However, they are also consistent with no significant repair below about ~ 0.5 Gy per fraction.

We conclude that dose sparing does continue to increase consistently with the LQ model, certainly at doses down to 1.4 Gy, and probably down to 0.8 Gy per fraction. No significant further sparing would be expected below 0.3 Gy per fraction in any case.

Before mentioning any clinical application, we wish to emphasize that a sufficient interval between fractions must occur, so as to allow full repair of sublethal injury. This requirement may conflict with the need to use hyperfractionated irradiation and the wish to avoid prolonging overall times. In the present experiments the minimum interval was 6 h, with alternate intervals 18 h. Experiments are under way to investigate the effect of shorter intervals.

If the LQ model is valid and an α/β value of 3 Gy can be assumed to apply down to 0.5 Gy per fraction, then considerably higher doses would be

tolerated by lung than the conventional $20 \times 2\,Gy = 40\,Gy$ total dose (Deeley, 1966). For example $50 \times 1\,Gy$, or $66 \times 0.8\,Gy = 52.8\,Gy$, or even $114 \times 0.5\,Gy = 57\,Gy$ should all give equivalent effects in lung, and greater effects on tumours with higher values of α/β.

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